Remarks

A. *Status of the Claims*

Claims 67-68, 73, 100-102, and 104-134 were pending at the time of the Action. Claims 67, 100, 110, 116, and 125 have been amended. Claims 108-109, 111-115, 121-124, and 126-134 have been canceled. Thus, claims 67-68, 73, 100-102, 104-107, 110, 116-120, and 125 are now pending. No new matter was added by these amendments.

B. *The Claims are Enabled*

The Action rejects claims 67-68, 73, 100-102, and 104-134 under 35 U.S.C. § 112, first paragraph, for lack of enablement. Applicants traverse this rejection.

There are a number of recurrent deficiencies in the Office Actions that have issued to date that Applicants would like to resolve. These are addressed in section B.1. entitled "Protein Therapy." A discussion of the Action's interpretation of "treatment" and "cure" is provided in section B.2. An analysis of the *Wands* factors is provided in section B.3.

1. Protein Therapy

A common issue that arises during the Action's analysis of the specification in accordance with the *Wands* factors is the alleged unpredictability of protein therapy. As discussed below, however, this alleged unpredictability is based on generalizations or factual misconceptions about protein therapy that are not an issue with the currently claimed method.

a) "effective protein production at the target site"

The Action states that one consideration for *in vivo* protein therapy is "effective protein production at the target site" (Action, p. 4). This, however, is not a consideration for the currently claimed method because it is an ACE2 ***polypeptide*** that is being administered to the mammal. Thus, protein production is not required at the target site.

b) "the art of protein therapy ... was unpredictable wherein protein is expressed in an individual suffering from cardiovascular or lung disorder"

The Action states that "the art of protein therapy ... was unpredictable wherein protein is expressed in an individual suffering from cardiovascular or lung disorder" (Action, p. 5-6). As explained in the section above, this is not a consideration for the currently claimed method because it is an ACE2 ***polypeptide*** that is being administered to the mammal. Thus, the alleged unpredictability of expressing the therapeutic protein in the individual is not an issue.

c) "providing the ACE2 in deficient cells in amount sufficient to treat genus of diseases associated with ACE2 decreased state by administering ACE2 polypeptide to any site"

The Action asserts that the specification fails to provide sufficient guidance regarding "providing the ACE2 in deficient cells in amount sufficient to treat genus of diseases associated with ACE2 decreased state by administering ACE2 polypeptide to any site" (Action, p. 4). Similar statements regarding the ability to increase the level of ACE2 ***in*** any cell are also made on pages 5, 6, and 10. ACE2 is a transmembrane protein, and the active ACE2 enzyme is secreted from cells by cleavage N-terminal to the transmembrane domain (*see* Donoghue *et al.* (2000), IDS ref. C13). Accordingly, ACE2 polypeptides are effective in circulation and need not be delivered ***into*** cells. Thus, someone of ordinary skill in the art would understand that the ACE2 polypeptide could be administered according to the teachings on at least pages 21-22 of the specification. The Neu and Schuster Declarations further demonstrate that an effective amount of ACE2 polypeptide can be delivered by intravenous injection or intraperitoneal injection, two routes of administration disclosed on page 21 of the specification.

d) "deletion of an individual gene ... may prove so drastic or so widespread as to create an amalgam of phenotypes whose interpretation becomes confounded by the interaction of various new physiologic changes"

In the paragraph bridging pages 6-7 in the Action, the Examiner states that deletion of an individual gene in a knockout animal may prove so drastic or so widespread as to create an amalgam of phenotypes whose interpretation becomes confounded by the interaction of various new physiologic changes. The Action, therefore, questions the interpretation of the phenotypes observed in the ACE2 knockout mouse reported in the specification. However, in addition to the ACE2 knockout mouse, the specification also describes an ACE/ACE2 double-knockout mouse. In the ACE/ACE2 double-knockout mice, it was shown that ablation of ACE expression on an ACE2 deficient background ***abolished*** the heart failure phenotype of ACE2 single-knockout mice (specification p. 29, first paragraph and p. 38, last paragraph). This shows that only the expected ACE2 activity on the polypeptides of the RAS systems (angiotensin I and angiotensin II conversion) was observed in the animal model. This refutes the Action's assertion that the interpretation of the results from this animal model could have been confounded by an amalgam of phenotypes and/or compensatory systems.

e) U.S. Patent 6,632,830 Incorrectly Predicted the Function of ACE2 Based on Its Homology to ACE

The Action cites Acton *et al.* (US 6,632,830) as providing a contradictory teaching as to the function of ACE2. As discussed in the specification and in previous responses, Acton incorrectly ***predicted*** a function for ACE2 based on its homology to ACE. No actual studies of ACE2 function were reported by Acton. Acton's prediction was proven wrong by ***actual studies*** disclosed in the present specification. The Action's continued reliance on Acton is unfounded.

f) Summary

As set forth above, much of the Action's enablement rejection is based factual deficiencies and/or generalizations about protein therapy that are not applicable to the currently claimed method. Applicants, therefore, respectfully request reconsideration of the enablement rejection in view of the comments above.

2. Treatment vs. Cure

The Action states that the difference between the Applicants' arguments and the Examiner's arguments appears to be the "therapeutic component" and the specificity of the method (Action, p. 10). Specifically, the Action contends that a therapeutically effective amount of soluble rhACE2 from a specific source used for treatment amounts to claiming a "cure." Equating "treatment" and "cure" in this manner is inconsistent with the teachings in the specification and the plain meaning of these terms.

The specification explicitly defines the term "treatment" as:

> an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treating" can also mean prolonging survival as compared to expected survival if not receiving treatment.

Specification, p. 10, ln. 1-9. A dictionary definition of "treatment" is "[a]n administration or application of remedies to a patient or for a disease or an injury;" whereas a dictionary definition for "cure" is "[r]estoration of health; recovery from disease." *The American Heritage Stedman's Medical Dictionary* (Houghton Mifflin Co. 2002). Thus, it is clear from both the definition in the specification and the definitions in the medical dictionary that while a method of "treatment"

may result in a "cure," it is not required that the treatment result in a cure of the disease. Thus, the Action's equating of "treatment" and "cure" is legally improper because it is inconsistent with the teachings in the specification and the plain meaning of these terms.

With respect to the Action's statement regarding the use of soluble rhACE2 from a specific source, Applicants note the following: (1) the active ACE2 enzyme was known to be a ***secreted*** protein at the time the present application was filed (*see e.g.,* Donoghue *et al.*, Circulation Research (2000) (IDS ref. C13)); (2) ACE2 is conserved among mammals, as well as other organisms (*see e.g.,* Specification, FIGs. 1A, showing an alignment of the amino acid sequences of ACE2 from human, rat, and mouse, which illustrates the identities and similarities between these sequences; p. 30, ln. 28 to p. 31, ln. 2, describing a study indicating that ACE/ACE2 functions in the heart have been conserved in flies; (3) a BLAST search of the ACE2 substrate, AngII, shows that AngII is present in numerous mammals including *Pan troglodytes, Mus musculus, Homo sapiens, Callithrix jacchus, Gorilla gorilla, Canis familiearis, Macaca mulatta, Rattus norvegicus, Ovis ammon,* and *Pongo pygmaeus*; and (4) a recombinant human ACE2 protein was able to treat mice and pigs (*see* Imai *et al.*, Neu Declaration, and Schuster Declaration). Accordingly, the recitation of "a mammalian ACE2 polypeptide" in the current claims is appropriate.

3. Analysis of the *Wands* Factors

The Action asserts that an analysis of the specification in accordance with the *Wands* factors demonstrates that it would require undue experimentation for a person of ordinary skill in the art to practice the claimed invention. However, contrary to the Action's assertions, an analysis of the *Wands* factors demonstrates that a person of ordinary skill in the art could make and use the claimed invention without undue experimentation.

As discussed in detail below, a person of ordinary skill in the art could make and use the claimed invention without undue experimentation in view of:

- the rat and mouse animal model studies in the specification showing the role of ACE2 in cardiac, lung, and kidney diseases;
- the disclosed similarities of the rat, mouse, and human ACE2 structure and function;
- the teaching in the specification that an ACE2 decreased state, such as cardiac, lung, and/or kidney disease, may be treated by administering to an animal in need thereof a therapeutically effective amount of an ACE2 polypeptide.

The enablement of the claims is further confirmed by:

- the demonstration by Imai *et al.* that injecting ACE2 knockout mice or acid-treated wild-type mice with a human ACE2 protein protected the mice from severe acute lung injury (*see* Neu Declaration, para. 14);
- the showing of a therapeutic benefit of administration of a human ACE2 polypeptide in a porcine ARDS model (*see* Neu Declaration, para. 11; Schuster Declaration, para. 17-37);
- the showing of a therapeutic benefit of administration of a human ACE2 polypeptide in a porcine pulmonary hypertension model (Schuster Declaration, para. 14);
- the showing of a therapeutic benefit of administration of a human ACE2 polypeptide in a mouse cardiovascular disease model (Schuster Declaration, para. 13-14); and
- the showing of a therapeutic benefit of administration of a human ACE2 polypeptide in a mouse kidney disease model (Schuster Declaration, para. 15-16).

In view of these results from ***two*** different mammalian species administered an ACE2 polypeptide from a ***third*** mammalian species to treat m***ultiple*** ACE2 decreased states, it is clear that the full scope of the claimed invention is enabled.

1. The Breadth of the Claims and the Nature of the Invention

Current claim 67 is directed to a method of treating an ACE2 decreased in a mammal, wherein the ACE2 decreased state is cardiovascular disease, renal failure, and/or lung disease, comprising identifying a mammal having cardiovascular disease, renal failure, and/or lung disease, and administering to the mammal a therapeutically effective amount of a mammalian

ACE2 polypeptide, wherein the cardiovascular disease, renal failure, and/or lung disease is treated.

2. The State of the Art and the Level of Ordinary Skill in the Art

The use of protein therapy in the treatment of diseases is well-known in the medical field (Neu Declaration, para. 6). Examples of such protein therapies are described in the publication *Scientific Considerations Related to Developing Follow-On Protein Products*, 2004, which is cited in the Action at page 9. This publication mentions such drugs as Epogen®, which is a ***protein therapy*** based on human erythropoietin; and Neupogen®, which is a ***protein therapy*** based on granulocyte colony-stimulating factor. As pointed out in the Action, *Scientific Considerations Related to Developing Follow-On Protein Products* also notes that ***six*** companies manufacture ***FDA-approved*** versions of human growth hormone (paragraph bridging pages 5-6). Thus, protein therapy was well-known and commercially successful in the art and the level of skill was high.

The Action notes that the half lives of six, FDA-approved human growth hormone drugs vary from 1.75 to 10 hours (Action, p. 9). From this, the Action concludes that "such large variations can impact the effectiveness of the product and the as [sic] the body's immune response to it." (Action, p. 9). The Action's conclusion is unsupported by the facts. ***All six*** of the human growth hormone products are FDA approved. Thus, the FDA determined that all six of the human growth hormone drugs were safe and effective even though their half lives varied from 1.75 to 10 hours. The Action's conclusion clearly does not follow from the facts.

Even though protein therapy in the treatment of diseases was well-known in the medical field, the Action asserts that the state of the art in protein therapy was unpredictable (Action, p. 5-6). In particular, the Action states that the artisan could not reasonably predict that any protein could be delivered in any cell of any mammal at therapeutically effective levels (Action, p. 5).

As explained in section B.1. above, the Action's position is based on generalizations or mischaracterizations of protein therapy that fail to consider the characteristics of the ACE2 protein. The active ACE2 enzyme is a ***secreted*** protein. This was known by those in the art at the time the present application was filed (*see e.g.,* Donoghue *et al.*, Circulation Research (2000) (IDS ref. C13)). Thus, the Action's concern with getting the protein into cells is unfounded. Moreover, the Action makes several references to the unpredictability of "expressing" the protein in the mammal. The reason for these statements is unclear because the current claims are directed to administering an ***ACE2 polypeptide***. Thus, these arguments fail to support the present rejection.

Furthermore, the evolutionary conservation of ACE2 structure and activity among mammals, as well as other organisms, is further evidence that the currently claimed method could be practiced in any mammal (*see e.g.,* Specification, FIGs. 1A, showing an alignment of the amino acid sequences of ACE2 from human, rat, and mouse, which illustrates the identities and similarities between these sequences). In addition, results in flies showed that a P-element mutation associated with the ACE homologue, ACER, results in a severe and lethal defect of heart morphogenesis providing further evidence that ACE/ACE2 functions in the heart have been conserved through evolution (Specification, p. 30, ln. 28 to p. 31, ln. 2). The previously cited publication entitled "Structure, Evolutionary Conservation, and Function of Angiotensin- and Endothelin-Converting Enzymes" (Macours *et al.*, *International Review of Cytology*, 239:47-97 (2004)), is further evidence of the conservation of the ACE/ACE2 system.

In addition, the specification discloses that AngI and AngII are substrates for ACE2, which functions as a carboxypeptidase to cleave a single residue from each of AngI and AngII (p. 2, ln. 5-10). Applicants also provided previously the results of a BLAST search of the ACE2

substrate, AngII, which shows that AngII is present in numerous mammals including *Pan troglodytes, Mus musculus, Homo sapiens, Callithrix jacchus, Gorilla gorilla, Canis familiearis, Macaca mulatta, Rattus norvegicus, Ovis ammon,* and *Pongo pygmaeus*. In view of the evidence that ACE2 structure and function is conserved among mammals, one would expect that the currently claimed method could be practiced in any mammal (*see* Neu Declaration, para. 5).

The Action cites Acton *et al.* (US 6,632,830) as providing a contradictory teaching as to the function of ACE2. However, Acton's prediction of the function of ACE2 was proven wrong by the studies disclosed in the present specification. Moreover, the studies in the specification have been confirmed by at least Imai *et al.*, the Neu Declaration, and the Schuster Declaration. The Action's continued reliance on Acton is improper.

The Action also undertakes a lengthy discussion of the unpredictability of using liposomes as delivery vehicles (Action, p. 8-9). Although the current claims use open claim language, and thus would encompass a method in which liposomes also were administered, there is no recitation of or requirement for liposomes in the current claims. An applicant need only provide an enabling disclosure for ***the claimed invention***. *In re Vaeck*, 947 F.2d 488, 496 (Fed. Cir. 1991). Moreover, liposomes were not used as delivery vehicles in the animal model studies described in the Neu and Schuster Declarations (discussed below). Thus, the Action's arguments regarding liposomes also fail to support the present rejection.

Furthermore, many of the Action's arguments and evidence of unpredictability pertain to safety and efficacy issues (*see* Action, p. 8-10). In particular, the Action points to references teaching the importance of dosing, clearance and efficacy of the product, preclinical evaluation for toxicity and immunogenicty. The MPEP states, however, that an applicant need not demonstrate that the invention is completely safe (MPEP § 2164.01(c)). Furthermore, testing for

the full safety and effectiveness of a particular drug for human use is more properly left to the Food and Drug Administration (FDA). *In re Brana*, 51 F.3d 1560, 1567 (Fed. Cir. 1995). "Usefulness in patent law, and in particular in the context of pharmaceutical inventions, necessarily includes the expectation of further research and development." *Id.* The stage at which an invention in the pharmaceutical field becomes useful is well before it is ready to be administered to humans. *Id.* Moreover, there is nothing in the patent statute or any other statutes that gives the Patent Office the right or the duty to require an applicant to prove that compounds he is claiming, and which he has stated are useful for "pharmaceutical applications," are safe, effective, and reliable for use with humans. *In re Krimmel*, 292 F.2d 948, 954 (C.C.P.A. 1961); *see also* MPEP § 2164.01(c).

The Examiner has the initial burden of producing reasons that substantiate a rejection based on lack of enablement. The Action's generalizations about clinical safety and efficacy fail to satisfy this burden. Furthermore, even if the Examiner had shifted the burden on enablement, the Examiner's allegations of unpredictability and undue experimentation are rebutted by the showing that a recombinant human ACE2 protein protected mice having a decreased ACE2 state from severe acute lung injury (Imai *et al.,* p. 112, col. 2; Figures 2(d)-(f)) in a study that employed the same ACE2 knockout mouse model described in the present specification, the same lung elastance assay described in the specification (*see* p. 40, ln. 12-20), and a route of administration (intraperitoneal injection) disclosed in the specification (*see* p. 21, ln. 27-30).

The Examiner's allegations of unpredictability and undue experimentation are also rebutted by: (1) the showing of a therapeutic benefit of administration of a human ACE2 polypeptide in a porcine ARDS model (*see* Neu Declaration, para. 11; Schuster Declaration, para. 17-37); (2) the showing of a therapeutic benefit of administration of a human ACE2

polypeptide in a porcine pulmonary hypertension model (Schuster Declaration, para. 14); (3) the showing of a therapeutic benefit of administration of a human ACE2 polypeptide in a mouse cardiovascular disease model (Schuster Declaration, para. 13-14); and (4) the showing of a therapeutic benefit of administration of a human ACE2 polypeptide in a mouse kidney disease model (Schuster Declaration, para. 15-16), which are discussed below.

As stated in the Neu Declaration: "Since ACE2 is an endogenous protein in mammals and the present specification discloses the physiological role of ACE2, it would require only routine clinical studies to administer a therapeutically effective amount of an ACE2 polypeptide to an mammal having hypertension, congestive heart failure, chronic heart failure, acute heart failure, myocardial infarction, arteriosclerosis, renal failure, and/or lung disease, in order to treat the mammal as recited in the current claims." (Neu Declaration, para. 6). This was demonstrated to be the case in the five different animal models mentioned above.

3. The Guidance Provided by the Specification

The present specification provides sufficient guidance to enable a person of ordinary skill in the art to make and use the claimed invention without undue experimentation. This is further supported by the studies described in the Neu and Schuster Declarations, which provide evidence that, by following the teachings in the specification, someone skilled in the art can make and use the claimed invention without undue experimentation.

The present specification provides a new paradigm for the regulation of the renin-angiotensin system. The present specification discloses that hypertension, as well as other cardiac, lung, and kidney diseases are associated with an ACE2 decreased state (*see e.g.,* p. 2, ln. 28 to p. 3, ln. 6). In particular, the rat and mouse studies in the present specification demonstrate that an ACE2 decreased state is associated with cardiovascular, renal, and lung diseases. For example, decreased ACE2 mRNA and protein levels were observed in the kidneys of

hypertensive rats (Specification, p. 32, ln. 21 – p. 33, ln. 22). In studies on the ACE2 knockout mouse, it was observed that loss of ACE2 leads to detrimental effects in the kidneys (p. 36, ln. 8-11), heart defects (p. 36, ln. 14 – p. 38, ln. 12), and increases the susceptibility of the lungs to injury (p. 40, ln. 12-20).

The specification further showed in the ACE/ACE2 double knockout mice, that ablation of ACE expression on an ACE2 deficient background ***abolished*** the heart failure phenotype of ACE2 single knockout mice (specification p. 29, first paragraph and p. 38, last paragraph). This shows that only the expected ACE2 activity on the polypeptides of the RAS systems (angiotensin I and angiotensin II conversion) was observed in the animal model. This refutes the Action's assertion that the interpretation of the results from this animal model could have been confounded by an amalgam of phenotypes and/or compensatory systems (Action, p. 6-7).

Accordingly, those of ordinary skilled in the art would have appreciated the therapeutic benefit of a method of treating an ACE2 decreased in a mammal, wherein the ACE2 decreased state is cardiovascular disease, renal failure, and/or lung disease, comprising identifying a mammal having cardiovascular disease, renal failure, and/or lung disease, and administering to the mammal a therapeutically effective amount of a mammalian ACE2 polypeptide, wherein the cardiovascular disease, renal failure, and/or lung disease is treated.

Moreover, enablement must be assessed from the position of a person of ordinary skill in the art. Acton *et al.* (US 6,194,556), which is cited in present specification (p. 2, ln. 11) and in the Action (*see e.g.,* p. 11), shows that ACE2 was fully available to those in the art at the time the present application was filed as a nucleotide sequence, as well as in vectors and plasmids for ACE2 expression (*e.g.* '556 patent, column 29, lines 15 to column 30 line 52). Polypeptides of ACE2 are disclosed in the '556 patent from column 30 to column 37 line 26. In addition,

pharmaceutical preparations and formulations of ACE2 are disclosed in the '556 patent at column 61 line 37 to column 36 line 37. The present specification on page 18, third paragraph, even cites to the '556 patent's corresponding Canadian patent CA 2,372,387 as describing methods by which ACE2 may be produced. A further document cited in the present specification on page 18, third paragraph, is Nichols *et al.* (JBC 277 (17):14838-14843 (2002)). Nichols shows an assay for ACE2 activation based on its proteolytic activity on small peptides. Thus, those of ordinary skill in the art would have been able to make and use ACE2 polypeptides without undue experimentation in view of the teachings in the specification and the knowledge in the art.

4. The Neu Declaration

As discussed in the previously submitted declaration of Dr. Nikolaus Neu ("Neu Declaration"), Imai *et al.* (*Nature*, 436:112-116 (2005); IDS reference C61)) is further evidence that those of skill in the art can make and use the claimed invention without undue experimentation (*see* Neu Declaration, para. 7). Imai *et al.* demonstrated that injecting ACE2 knockout mice or acid-treated wild-type mice with a recombinant human ACE2 protein protected the mice from severe acute lung injury (p. 112, col. 2; Figures 2(d)-(f)). This study employed the same ACE2 knockout mouse model described in the present specification, the same lung elastance assay described in the specification (*see* p. 40, ln. 12-20), and a route of administration (intraperitoneal injection) disclosed in the specification (*see* p. 21, ln. 27-30). Thus, Imai *et al.* demonstrate that those of skill in the art can treat an ACE2 decreased state by administering a therapeutically effective amount of an ACE2 polypeptide (Neu Declaration, para. 7). The results of Imai *et al.* also provide additional evidence of the conserved function of ACE2 and the renin-angiotensin system by virtue of the fact that a human ACE2 protein was able to complement ACE2 function in mice (Neu Declaration, para. 7).

In another study described in the Neu Declaration, recombinant human soluble ACE2 (rhACE2) protein was studied in a piglet acute respiratory distress syndrome (ARDS) model (Neu Declaration, para. 8). The study was conducted by Alexander Löckinger and Benedikt Treml of Dr. Neu's research group, with the pharmacological evaluation being carried out by Manfred Schuster and Hans Loibner of Apeiron Biologics (Neu Declaration, para. 8). In this study, an ACE2 polypeptide, rhACE2, was administered as a central venous bolus injection at a dose of 100 μg/kg following the last LPS bolus injection and 120 minutes from the start of the continuous LPS infusion (Neu Declaration, para. 9). Intravenous injection is a route of administration disclosed in the present specification (*see* Specification, p. 21, ln. 27-30; Neu Declaration, para. 9). The rhACE2 bolus injections were well tolerated and did not show any apparent side effects (Neu Declaration, para. 9). Treatment with rhACE2 stabilized or even decreased slightly pulmonary arterial pressure (PAP), while the control group showed a nearly 15% increase in PAP (Neu Declaration, para. 11). Systolic arterial pressure (SAP) was also measured. The control group showed an increase in SAP up to 12%, whereas after rhACE2 injection a stabilization and 5% decrease in SAP was observed (Neu Declaration, para. 11). The difference between the control and rhACE2 treatment groups was significant (Neu Declaration, para. 11).

In addition, oxygen concentration was measured in arterial and venous blood samples taken from the piglets every 30 minutes (Neu Declaration, para. 12). There was a potential stabilization observed of arterial as well as venous oxygen concentration in the group receiving rhACE2, however the data did not reach statistical significance in this study and will have to be confirmed in further experiments (Neu Declaration, para. 12). The results of this study also

provide additional evidence of the conserved function of ACE2 and the renin-angiotensin system by virtue of the fact that a human ACE2 protein was used to treat pigs.

In view of the *in vivo* rat and mouse data on the role of ACE2 in cardiac, lung, and kidney diseases; the disclosed similarities of the rat, mouse, and human ACE2 sequences; and the knowledge in the art of ACE2 sequences, expression constructs, and formulations; those of ordinary skill in the art could have practiced the claimed method in a multitude of mammals including humans. This is confirmed by the studies described in Imai *et al.* and in the Neu Declaration.

5. The Schuster Declaration

As further evidence of the enablement of the current claims, Applicants previously provided the declaration of Dr. Manfred Schuster ("Schuster Declaration"). Dr. Schuster is the Head of Research and Development at Apeiron Biologics, which is the licensee of the present patent application. The Schuster Declaration describes four studies on therapeutic uses of recombinant human soluble ACE2 (rhACE2) protein performed under Dr. Schuster's direction at Apeiron Biologics and in collaboration with researchers at University Hospital Innsbruck (Schuster Declaration, para. 2). Specifically, these studies describe the use of rhACE2 in treating: (1) cardiovascular complications in mice; (2) pulmonary hypertension in pigs; (3) kidney disease in mice, and (4) acute respiratory distress syndrome in pigs (Schuster Declaration, para. 2).

The Schuster Declaration states that the studies of the rhACE2 protein were pursued because of the disclosure in the present specification that ACE2 was a critical negative regulator of the renin-angiotensin system (RAS) and that the activation of ACE2 could be used to treat hypertension, cardiac disease, kidney disease, and lung disease (Schuster Declaration, para. 4). Based on teachings in the specification, a recombinant human ACE2 (rhACE2) protein was

produced and provided in a physiological buffer for use in these studies (Schuster Declaration, para. 5 and 6). The recombinant human ACE2 protein used in the studies is referred to interchangeably in the Schuster Declaration as rhACE2 and APN 01 (Schuster Declaration, para. 5).

a) Cardiovascular Complications in Mice

The Schuster Declaration states that by following the teachings in the present specification it was demonstrated that rhACE2 can treat cardiovascular complications in mice (Schuster Declaration, para. 5). In particular, the Schuster Declaration (para. 7) noted teachings in the specification that: (1) ACE2 is a critical negative regulator of the renin-angiotensin system (RAS) (Specification, paragraph bridging pages 2-3); (2) ACE2 cleaves angiotensin I (Ang I) to generate Ang 1-9, and it cleaves angiotensin II (Ang II) to generate Ang 1-7 (Specification, p. 29, ln. 10-16) (*see also* Exhibit 2, Figure 1); (3) loss of ACE2 resulted in an increase in Ang II and led to detrimental heart defects in the ACE2 knockout mouse (Specification, p. 36, ln. 14 – p. 38, ln. 26); (4) an ACE2 decreased state, such as cardiac disease, may be treated by administering to an animal in need thereof effective amount of an agent that can increase the expression of ACE2 (Specification, p. 9, ln. 10-15); (5) the agent that increases the expression of ACE2 may be an ACE2 protein (Specification, p. 9, ln. 16-25); and (6) the agent may be administered to a subject via intravenous injection or intraperitoneal injection (Specification, p. 21, ln. 27-30). Accordingly, rhACE2 was administered either by intravenous injection or intraperitoneal injection to: (1) healthy Balb-c mice, (2) healthy Balb-c mice administered Ang II, (3) Balb-c mice and ACE2 knock-out Balb-c mice subjected to aortic banding heart failure, and (4) Balb-c mice subjected to coronary ligation ischemia (Schuster Declaration, para. 7).

The results of the study using rhACE2 to treat cardiovascular complications in mice showed that ACE2 therapy reduced heart beat rate and increased heart efficiency, neutralized or significantly reduced the negative effects of elevated Ang II, relieved the symptoms of aortic banding in ACE2 knock-out mice, and provided a therapeutic benefit in myocardial infarction (Schuster Declaration, para. 12; *see also* para. 8-11).

b) Pulmonary Hypertension in Pigs

The Schuster Declaration states that by following the teachings in the present specification it was demonstrated that rhACE2 can treat pulmonary hypertension in pigs. In particular, the Schuster Declaration (para. 13) noted teachings in the specification that: (1) ACE2 cleaves angiotensin I (Ang I) to generate Ang 1-9, and it cleaves angiotensin II (Ang II) to generate Ang 1-7 (Specification, p. 29, ln. 10-16) (*see also* Exhibit 2, Figure 1); (2) the specification discloses that ACE2 is expressed in the lung (Specification, p. 40, ln. 11-12); (3) loss of ACE2 resulted in increased sensitivity to lung injury in the ACE2 knockout mouse (Specification, p. 36, ln. 14 – p. 38, ln. 26); (4) an ACE2 decreased state, such as lung disease, may be treated by administering to an animal in need thereof an effective amount of an agent that can increase the expression of ACE2 (Specification, p. 9, ln. 10-15; p. 40, ln. 18-20); (5) the agent that can increase the expression of ACE2 can be an ACE2 protein (Specification, p. 9, ln. 16-25); and (5) the agent may be administered to a subject via intravenous injection (Specification, p. 21, ln. 27-30). Accordingly, the effects of rhACE2 administered by intravenous injection to piglets ventilated using a hypoxic gas mixture were studied (Schuster Declaration, para. 13).

The results of this study demonstrated that the treatment was well tolerated without any signs of side effects or toxicity (Schuster Declaration, para. 14). In addition, the results indicated a therapeutic benefit of administering ACE2 in pulmonary hypertension as evidenced by the

significant decrease in mean pulmonary arterial pressure in animals treated with rhACE2 (Schuster Declaration, para. 14).

c) Kidney Disease in Mice

The Schuster Declaration states that by following the teachings in the present specification it was demonstrated that rhACE2 can treat diabetic nephropathy in mice (Schuster Declaration, para. 15). In particular, the Schuster Declaration (para. 15) noted teachings in the specification that: (1) ACE2 is expressed in the kidney (Specification, p. 35, ln. 9-10); (2) loss of ACE2 resulted in enhanced Ang II signaling which ultimately mediated detrimental effect in the kidneys of the ACE2 knockout mouse (Specification, p. 35, ln. 9 to p. 36, ln. 11); (3) an ACE2 decreased state, such as kidney disease, may be treated by administering to an animal in need thereof an effective amount of an agent that can increase the expression of ACE2 (Specification, p. 9, ln. 10-15); (4) the agent that can increase the expression of ACE2 may be an ACE2 protein (Specification, p. 9, ln. 16-25); and (5) the agent may be administered to a subject via intraperitoneal injection (Specification, p. 21, ln. 27-30). Accordingly, rhACE2 was administered by intraperitoneal injection to mice with diabetic nephropathy (Schuster Declaration, para. 15 and 16).

Kidney function was assessed by measuring albumin excretion in urine (Schuster Declaration, para. 16). The results of this study showed that albumin excretion in urine due to kidney damage was reduced significantly compared to a control group following 4 weeks of treatment with rhACE2 (Schuster Declaration, para. 16). These findings confirm a therapeutic benefit of ACE2 in diabetic nephropathy (Schuster Declaration, para. 16).

d) Acute Respiratory Distress in Pigs

The Schuster Declaration also provides an update on the study previously described in the Neu Declaration. As noted in both the Schuster Declaration and the Neu Declaration, this

study is a collaboration between researchers at Apeiron Biologics and University Hospital Innsbruck. The ARDS animal model provides reproducible conditions in which to evaluate the effects of administrated drugs (Schuster Declaration, para. 36). A variety of pharmacological and physiological parameters were evaluated over the course of the study (*see* Schuster Declaration, para. 18-36). The results of the study in the ARDS piglet model demonstrate the ACE2 therapy is well tolerated and provides a therapeutic benefit (Schuster Declaration, para. 25 and 37). In particular, the study demonstrated that ACE2 therapy increased lung function and improved kidney function in the ARDS piglet model (Schuster Declaration, para. 37; *see also* para. 18-36).

Further studies in the ARDS piglet model were performed to further elucidate the mechanism behind the therapeutic effects of ACE2 treatment (Schuster Declaration, para. 49). The drug Telmisartan, which blocks AngII signaling via the AT1 receptor, was administered alone or in combination with rhACE2 to see if the therapeutic effects of the ACE2 treatment were related to reduced Ang II signaling via AT1 receptor or if other, AT1-independent effects, were responsible for the therapeutic benefit (Schuster Declaration, para. 49). The Schuster Declaration reports that the therapeutic benefit of an ACE2 therapy does not appear to be mediated only by the reduction of AT1 related signaling caused by lowered Ang II titers (para. 49).

e) Summary

In summary, the Schuster Declaration shows that by following the teachings in the present specification, one can make and use the full scope of the claimed invention without undue experimentation. In particular, the Schuster Declaration demonstrates that a therapeutically effective amount an ACE2 polypeptide has a beneficial effect in treating: (1) cardiovascular complications, (2) pulmonary hypertension, (3) kidney disease, and (4) acute

respiratory distress syndrome (Schuster Declaration, para. 51). Moreover, these beneficial effects were demonstrated in two mammalian species, mice and pigs (Schuster Declaration, para. 51). Additionally, the beneficial effects achieved in mice and pigs resulted from administering a human ACE2 polypeptide (Schuster Declaration, para. 51). Thus, in view of these results from two different mammalian species administered an ACE2 polypeptide from a third mammalian species, the Schuster Declaration states that one would expect that ACE2 decreased states could similarly be treated in any mammal (Schuster Declaration, para. 51). As discussed above, this is also confirmed by the studies described in Imai *et al.* and in the Neu Declaration.

6. The Existence of Working Examples

The Action asserts that the working examples in the specification demonstrate the role of ACE2, but do not demonstrate any method of treating any condition by administering any composition of ACE2. Compliance with the enablement requirement, however, turns on whether the invention is disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation. MPEP § 2164.02. As discussed above, the present invention provides working examples showing that an ACE2 decreased state is associated with cardiovascular, renal, and lung diseases (*see* Specification, p. 32, ln. 21 – p. 33, ln. 22; p. 36, ln. 8 – p. 38, ln. 12; p. 40, ln. 12-20). Accordingly, those of ordinary skilled in the art would have appreciated the therapeutic benefit of treating an ACE2 decreased state by administering to a mammal a therapeutically effective amount of an ACE2 polypeptide. The specification provides the sequences of human, rat, and mouse ACE2 DNA and polypeptides (FIG. 1a, 10, and 11). The specification also discloses routes of administration and dosages at, for example, page 21, line 27 to page 22, line 17.

As described in the preceding sections, Imai *et al.* demonstrated that one skilled in the art is be able to practice the claimed invention without an undue amount of experimentation by

demonstrating that a recombinant human ACE2 protein protected mice having a decreased ACE2 state from severe acute lung injury (p. 112, col. 2; Figures 2(d)-(f)) in a study that employed the same ACE2 knockout mouse model described in the present specification, the same lung elastance assay described in the specification (*see* p. 40, ln. 12-20), and a route of administration (intraperitoneal injection) disclosed in the specification (*see* p. 21, ln. 27-30) (*see also* Neu Declaration, para. 7). The enablement of the currently claimed invention is further confirmed by the study in the piglet ARDS model discussed above and described in the Neu Declaration (para. 8-12) and Schuster Declaration (para. 17-37) and in the mouse cardiovascular disease model and the mouse kidney disease model described in the Schuster Declaration at paragraphs 13-14 and 15-16.

7. Summary

In view of the rat and mouse animal model studies in the specification showing the role of ACE2 in cardiac, lung, and kidney diseases; the disclosed similarities of the rat, mouse, and human ACE2 structure and function; and the teaching in the specification that an ACE2 decreased state, such as cardiac, lung, and kidney disease, may be treated by administering to an animal in need thereof an effective amount of an agent that can increase the expression of ACE2; a person of ordinary skill in the art could make and use the currently claimed invention without undue experimentation (*see* Neu Declaration, para. 14; *see also* Schuster Declaration, para. 51). The enablement of the claims is confirmed by the demonstration by Imai *et al.* that injecting ACE2 knockout mice or acid-treated wild-type mice with a rhACE2 protein protected the mice from severe acute lung injury; and the study in the piglet ARDS model showing that rhACE2 protein therapy stabilized or even decreased both pulmonary arterial pressure and systolic arterial pressure (*see* Neu Declaration, para. 14). The enablement of the claims is further confirmed by the studies described in the Schuster Declaration, which demonstrate that a therapeutically

effective amount an ACE2 polypeptide has a beneficial effect in treating cardiovascular complications, pulmonary hypertension, kidney disease, and acute respiratory distress syndrome. The Schuster Declaration concludes that in view of these results from two different mammalian species administered an ACE2 polypeptide from a third mammalian species, one would expect that ACE2 decreased states could similarly be treated in any mammal (Schuster Declaration, para. 51).

The current claims are, therefore, enabled. Thus, Applicants respectfully request the withdrawal of this rejection.

C. *The Claims are Definite*

1. Claims 67-68, 73, and 100-107

The Action rejects claims 67-68, 73, 100-102, and 104-134 under 35 U.S.C. § 112, second paragraph, as allegedly omitting essential steps. Specifically, the Action asserts that for claims 67-68, 73, 100-102, and 104-107, the missing essential step is a step of determining the decreased state of ACE2. For claims 108-134, the Action asserts that the missing step is a step to indicate how the disease is controlled. Applicants traverse this rejection.

Current claim 67 recites that the ACE2 decreased state is a cardiovascular disease, renal failure, and/or lung disease. Claim 67 also recites the step of identifying a mammal having cardiovascular disease, renal failure, and/or lung disease. This identification is sufficient to identify the ACE2 decreased state because hypertension, as well as other cardiac, lung, and kidney diseases are associated with an ACE2 decreased state (*see e.g.,* p. 2, ln. 28 to p. 3, ln. 6). The involvement of ACE2 in these diseases is related to its role as a negative regulator in the renin-angiotensin system (RAS) (*see* Specification, paragraph spanning pages 2-3). The RAS is a regulator of blood pressure homeostasis (Specification, p. 1, ln. 21-22). The enzyme ACE cleaves AngI to AngII, which can contribute to hypertension by promoting vascular smooth

muscle vasoconstriction and renal tubule sodium reabsorption (Specification, p. 1, ln. 23-27). ACE2, on the other hand, decreases AngII signaling because ACE2 cleaves AngI to Ang1-9 and AngII to Ang1-7 (Specification, p. 2, ln. 5-9; p. 29, ln. 8-16). Thus, ACE2 can be used to treat cardiovascular disease, renal failure, and lung disease, which are associated with vascular smooth muscle vasoconstriction and/or renal tubule sodium reabsorption.

Furthermore, determining the level of ACE2 in a subject prior to treatment is unnecessary. This is evident from the animal model studies in which mice with severe acute lung injury (see Neu Declaration, para. 14); pigs with ARDS (see Neu Declaration, para. 11; Schuster Declaration, para. 17-37); pigs with pulmonary hypertension (Schuster Declaration, para. 14); mice with cardiovascular disease (Schuster Declaration, para. 13-14); and mice with kidney disease (Schuster Declaration, para. 15-16), were all treated for their respective disease without first having the ACE2 level determined.

In view of the above, Applicants respectfully request the withdrawal of this rejection.

D. *The Claims Are Novel Over Acton*

Claims 67-68, 73, 100-102, and 104-134 are rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by Acton (U.S. Patent 6,194,556). Applicants traverse this rejection.

For a publication to anticipate a claim under 35 U.S.C. § 102 it must not only disclose all elements of the claim within the four corners of the document, but must also disclose those elements ***"arranged as in the claim."*** *Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1369 (Fed. Cir. 2008). The Action, however, has not identified any disclosure in Acton that even suggests using ***ACE2*** to treat cardiovascular, lung, and/or kidney disease. Rather, the Action merely cites to disclosures in Acton of various conditions and of various ACE2 therapeutics (ACE2 agonists or ACE2 antagonists) without any regard for which conditions Acton states could be treated with either an ACE2 agonist or antagonist.

As explained on page 2 of the Specification and in previous responses, Acton predicted that ACE2 functioned to hydrolyze AngI into AngII, which is a vasoconstrictor, based on its homology to ACE (Acton, col. 56, ln. 19-39). Accordingly, Acton predicted that ACE2 ***antagonists*** would be useful in treating hypertension and congestive heart failure (Id.; *see also* col. 7, ln. 47-51; col. 57, ln. 10-20). In other words, Acton incorrectly predicted a function for ACE2 that was the ***opposite*** of what it was later demonstrated to be. It appears that the only therapeutic uses of an ACE2 ***agonist*** contemplated by Acton were for the treatment of inflammation, burns, and insect bites (Acton, col. 58, ln. 7, ln. 51-54). The Action's mixing and matching of disparate teachings in Acton is legally flawed. *See e.g.,* 545 F.3d at 1369.

For the reasons above, the Action fails to establish a *prima facie* case of anticipation because the Action fails to show that Acton discloses all of the claim elements as arranged in the current claims. Applicants, therefore, respectfully request the withdrawal of this rejection.

E. *Conclusion*

Applicants believe that this is complete reply to the Office Action dated December 15, 2008. Should the Examiner have any questions, comments, or suggestions relating to this case, the Examiner is invited to contact the undersigned Applicants' representative at (512) 536-5654.

Respectfully submitted,

Travis M. Wohlers
Reg. No. 57,423
Attorney for Applicants

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 536-5654 (telephone)
(512) 536-3035 (facsimile)

Date: March 12, 2009